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Effect of Variable Salinity on the Growth Rate, Biomass Production, Pigment Composition and Lipid Yield in Halophilic Microalgae *Euglena* sp. (Euglenales : Euglenaceae)

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ABSTRACT

One of the major ecological factors limiting growth and productivity of most marine microalgae is salinity. High salt concentrations result in cell membrane damage, enzyme activity suppression, disturbances in vital functions like cell division, carbon assimilation, metabolism of proteins and nucleic acids, decreases in respiration intensity and accumulations of toxic products in cells. In the present investigation, the influence of salinity on growth rate, biomass production, pigments and lipid concentration of halophilic microalga *Euglena* sp. was investigated. The species was cultivated in the salinity varied from 30—55 PSU under optimized condition. The best growth rate, biomass and lipid concentration were obtained at 30 PSU indicated that isolated species prefer alkaline condition for their optimum growth. At higher salinity of 55 PSU, the growth rate, biomass and amount of lipid was drastically decreased. These factors are responsible to understand their response in varying environmental factors and also will produce high biomass and oil for biodiesel production. Lower saline conditions successfully applied to grow halophilic *Euglena* sp. in lab conditions to get the highest yield of biomass with high pigment content.

Keywords Halophilic microalgae, *Euglena* sp., Environmental factors, Pigments, Lipid.

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INTRODUCTION

Algal cells are generally able to live within a certain range of enhanced salt concentrations or changing salinities, since most probably all life originated in the oceans, i.e. a highly saline environment. Microalgae are a group of plankton that produce

biochemical products, include production of a wide array of carbohydrates, lipids and proteins that are commercially valuable (Rad et al. 2015). However, during evolution, the degree of salt resistance and salt tolerance became very divergent among the present day aquatic organisms. Numerous investigations of algal species have demonstrated that microalgae react to the changes in the ecosystem where they are grown (Valenzuela-Espinoza et al. 2002, Scragg 2002). The environmental factors like pH, temperature, light intensity and nutritional factors (nitrogen, phosphorus and iron) affect the growth, lipid yield, pigment composition of the alga. This behavior response is biotechnological characteristics for scientific industry and that can be controlled algal biochemical composition and development, concentrating on particular mixes and higher productivity (Chia et al. 2013, Rocha et al. 2003, Bano and Siddiqui 2004, Raghavan et al. 2008). Pigment content is based on the growth, biomass and nutrient concentration of phytoplankton cultivation (Gitelson et al. 1995). Salinity and temperature have been shown to induce the characteristic of the nutritional properties in microalgae (Hemaiswarya et al. 2011). A number of factors are known to influence the lipid content of microalgae, such as nitrogen (Illman et al. 2000) and silicon (Lynn et al. 2000) deficiency phosphate limitation, high salinity (Rao et al. 2007). The composition of intracellular lipid of microalgae was reported to change in response to environmental salinity. Increase of salinity concentration from 0.4 M to 4 M increased saturated and monounsaturated fatty acids in *Dunaliella* cells isolated from an antarctic hypersaline lake (Xu and Berdall 1997). Salt might have a direct effect upon processes involved in electron transport and/or photophosphorylation and result in a decreased quantum efficiency of photosynthesis. The present study was focus on the effect of variable range of salinity on biomass yield, growth and pigment content of unicellular flagellate *Euglena* sp.

MATERIALS AND METHODS

Halophilic microalgae and laboratory culture conditions

The halophilic microalgae used in the present study were collected from the saltpan located at Tuticorin, South-east coast of India (Lat.8°45' N and 78°13'E) using plankton net (mesh size 48 µm). *Euglena* sp. was isolated from hypersaline environment and culture was maintained in Marine Planktonology and Aquaculture Laboratory, Department of Marine Science, Bharathidasan University. Tiruchirappalli 24, Tamil Nadu, India. Stock cultures of *Euglena* sp. was maintained in conway's media (Walne 1970) and incubated for 20 days under controlled condition.

Growth of microalgae at various salinities

The halophilic microalgal study was determined using different salinity concentrations i.e., 30, 35, 40, 45 and 55 PSU for hypersaline microalgae. In this study the effect of salinity on the growth and lipid experiments were carried out in 250 ml Erlenmeyer flasks each containing 100 ml of appropriate medium incubated at 25°C under 12 : 12 light : dark (L : D) photoperiod. Control cultures were also maintained under optimized conditions. The experiments were extended up for 20 days. The growth rate, biomass, lipidand pigments yield were determined at each growth phases of microalgae.

Determination of chlorophyll pigment

A known aliquot of microalgal sample was centrifuged for 5 min and supernatant was discarded. The pellet was resuspended in the same amount of methanol and shaken thoroughly. The tubes were kept in hot water bath for 30 mins and centrifuged. The absorbance was measured at 665.2 nm ($A_{665.2}$) and 652.4 nm ($A_{652.4}$) and chlorophyll was estimated according to standard equation (Lichtenthaler 1987) as follows :

$$\text{Chlorophyll a (Chl-a, mg/l)} = 16.72\ A_{665.2} - 9.16\ A_{652.4}$$

$$\text{Chlorophyll b (Chl-b, mg/l)} = 34.09\ A_{652.4} - 15.28\ A_{665.2}$$

Determination of growth rate and biomass

Algal growth was measured by taking absorbance and biomass. Absorbance was measured on regular intervals (5,10,15 and 20 days respectively) by re-

cording the changes in optical density at 680 nm with a UV/ vis spectrophotometer. Gravimetrical method was used in the experiment to determine the dry cell weight of biomass. Centrifuge was used to extract the biomass from the known culture quantity. The extracted culture was then dried at 80°C to remove the moisture content (Rai and Abraham 1993) and below shown formula was used to calculate the biomass productivity (g l^{-1} d^{-1}).

$$\text{Biomass productivity} = \frac{X_1 - X_0}{\text{Number of days}}$$

Where, X_1 = Final concentration of biomass, X_0 = Initial concentration of biomass.

Extraction of total lipids

The total lipids were extracted by mixing chloroform–methanol (4 : 2 v/v) with the algal samples adopting the standard procedure of Folch et al. (1957). A mixture of 2 ml methanol and 1 ml chloroform was made and added to 1 g algal biomass. It was kept for 24 h at room temperature to dissolve the lipids properly. The mixture was centrifuged at 3000 rpm for 10 min. Supernatant was separated, 2 ml of chloroform was again added to the pellets and shaken properly. It was again centrifuged at 3000 rpm for 5 min and supernatant was separated. After adding 2 ml of 1% KCL to the supernatant, two layers were formed. Lower layer was pipette out and weighed. The percentage of lipid in algal sample was calculated using the following formula :

$$\text{Lipid content (\%)} = \text{wt.of lipid (g)} \times 100 / \text{wt.of culture (g)}$$

Statistical analysis

To validate the results, all the experiments were performed in triplicates. Further, they have been repeated twice to ensure the reproducibility of the results and the average values (mean ± SD) are listed.

RESULTS AND DISCUSSION

The effect of salinity on isolated *Euglena* species was determined by quantifying their growth rate, biomass, lipid and chlorophyll pigment yield. *Euglena* sp. grown on Conway's medium was sterilized and kept for 12 h light regime and 12 h dark regime and then incubated for 20 days. The growth, biomass, lipid and chlorophyll yield were calculated for each growth phase of algae. The results showed that more intracellular lipids started to accumulate in *Euglena* sp. on the stressed conditions, which increased the biomass, growth and chlorophyll concentration. The microscopic image of *Euglena* sp. was shown in Figure 1.

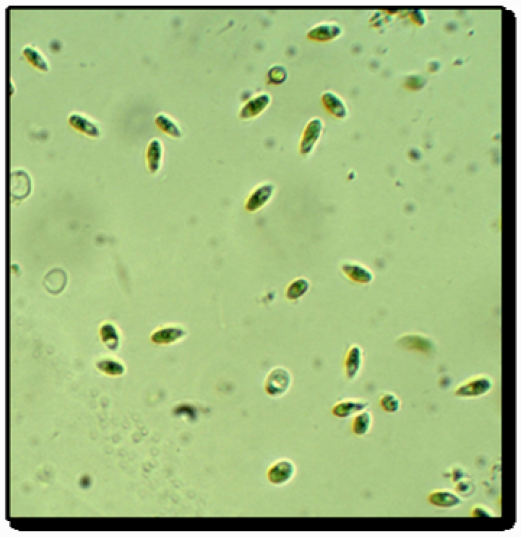

Fig. 1. Microscopic image of *Euglena* sp.

Effect of salinity on pigment production

Chlorophyll is the most abundant pigment present in green algae. Hence, it was suggested that salt stress in microalgae can be measured by quantitative estimation of chlorophyll pigments (Ahmad et al. 2016). Therefore, chlorophyll quantification was considered a growth responsive parameter and was correlated with growth kinetics associated with diverse *in vitro* conditions (Demetriou et al. 2007). Halophilic microalgae grown under different salinity conditions indicated that reduction in chlorophyll concentration and displayed similar trends with previous reports (Hiremath and Mathad 2010, Kirroliaa et al. 2011). Experiments conducted at varied salinity to study pigment accumulation in *Euglena* sp. which is shown in Figure 2a and 2b. In the present study

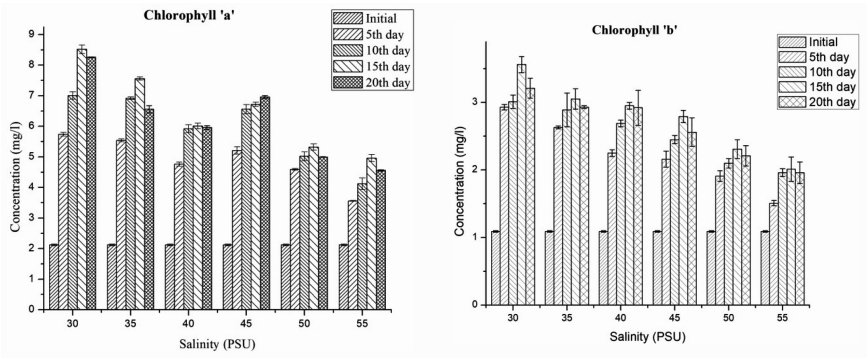

Fig.2. Effect of salinity on pigments production of *Euglena* sp. : (a) Chlorophyll a, (b) Chlorophyll b.

variable salinity range showed the increasing trend of pigment from 0th day to 15th day after which was declined at 20th day. The maximum concentration of chlorophyll a and b was observed in 30 PSU with concentration of 8.52 ± 0.14 and 3.56 ± 0.12 mg/l respectively and the minimal pigment concentration was observed in 55 PSU. The chlorophyll a and b decreased during each stage of the experiment when the salinity concentration increased. Similar results have been observed by Yeo and Flowers (2006). This is indirect evidence of the dependence of photochemical activity in microalgae cells on salt concentrations. It was observed that reduction in chlorophyll contents can be attributed to the modification in the metabolic system of microalgae. In the presence of these stress conditions, a small fraction of the absorbed light energy only enters into the photo system pathways and subsequently decreases the total quantum yield of pigments. These induced outcomes subsequently reduced the chlorophyll synthesis and biomass accumulation (Sudhir and Murthy 2004).

Effect of salinity on growth rate and biomass

Effect of salinity on cell density was studied by growing the *Euglena* sp. in media having varied salinity at ranged from 30 to 55 PSU. Growth parameters of *Euglena* sp. was studied in terms of cell density and biomass at specific time intervals. In the present study, the higher growth rate and biomass were observed in *Euglena* sp. with lower salinity of 30 PSU (Figs. 3 and 4). Changes in salinity also affect the photosynthetic rate of phytoplankton (Moisander et al. 2002, Lartigue et al. 2003, Rai et al. 2015). It has

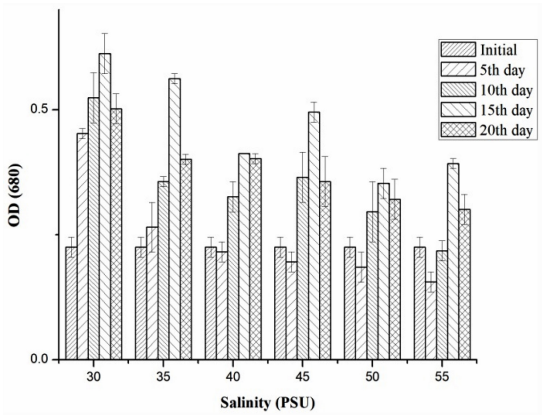

Fig. 3. Effect of salinity on growth rate and of *Euglena* sp.

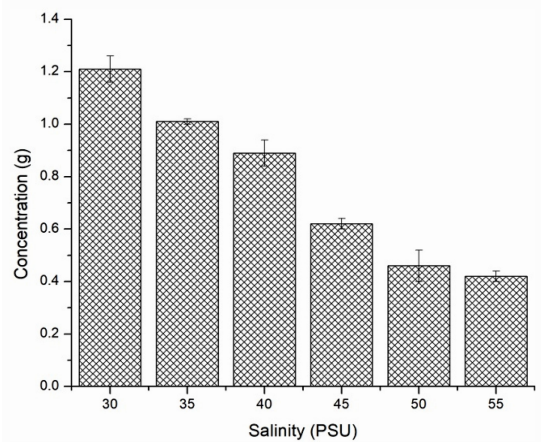

Fig. 4. Effect of salinity on biomass production of *Euglena* sp.

been reported previously that under salinity stress, microalgae expend most of their energy to maintain cell turgor pressure and to resist the osmotic stress rather than increase their growth which led to a reduction in biomass productivity (Kirst 1990). The gradual salinity increase not only affected biomass productivity, but also markedly affected cell volumes. Slow growth rate at high salinity resulted in low biomass productivity when cells grown outside of their optimum growth salinity range. It has been reported that increased salinity concentrations stimulate the Na^+ channel blocker and in response the cytoplasmic volume decreases (Allakhverdiev et al. 2000, Takagi et al. 2006). Smith and Berry (1986) suggested that hyperosmotic dehydration could be a reason for cell volume reduction at high salinity.

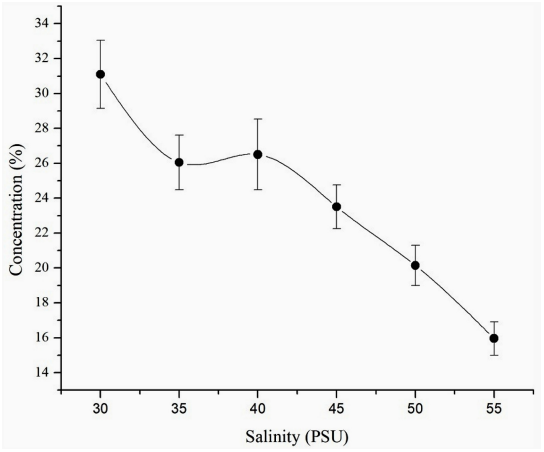

Fig. 5. Effect of salinity on lipid yield of *Euglena* sp.

Effect of salinity on lipid profile of microalgae

Numerous theories have been proposed to explain the mechanism of lipid accumulation in microalgae under various stress conditions (Solovchenko 2013). It was observed that various salts and their respective concentrations affect microalgae metabolism not only in terms of growth but also in lipid accumulation and modulation in lipid profile. In the presence of salt stress condition, microalgae system enhances the intracellular lipid that acts as a storage energy material till the favorable conditions are attained (Mohan and Devi 2014). The outcome of the present study highlights that the highest concentration of lipid was observed in lowest salinity (30 PSU) with the concentration of 31.01 ± 1.56% and then it decreased to the lowest level (15.96 ±0.42%) at 55 PSU after 20 days of cultivation which is shown in Figure 5. Abu-Rezq et al. (1999), Guschina and Harwood (2006) also have shown that lipid content of microalgae changes with chage in salinity. Griffiths and Harrison (2009) have found high lipid production in *Thalassiosira weissflogii* at a low salinity range. High lipid content at high and low salinity may be due to adaptation under stress conditions (Takagi et al. 2006). However, previous studies in literature also suggested the application of high salt concentration to induce the lipid accumulation (Xia et al. 2014).

CONCLUSION

The effect of various concentrations of salinity on the isolated alga *Euglena* sp. showed, increased growth rate and biomass yield at 30 PSU salinity concentration and then it subsequently decreases with increase in salinity concentration. While, chlorophyll and lipid content was also increased with decreased salinity. The microalgae *Euglena* sp. was considered to be a promising source of high value compounds for the pharmaceutical and food industry. They form a very important biofuel component in aquaculture practices due to their high content of lipid compounds. Thus it is essential to identify, isolate and mass cultivate monocultures under controlled laboratory conditions. The study on the effect of salinity on the growth of the isolates are useful for optimizing cell growth and is necessary to understand their response to varying environmental factors.

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